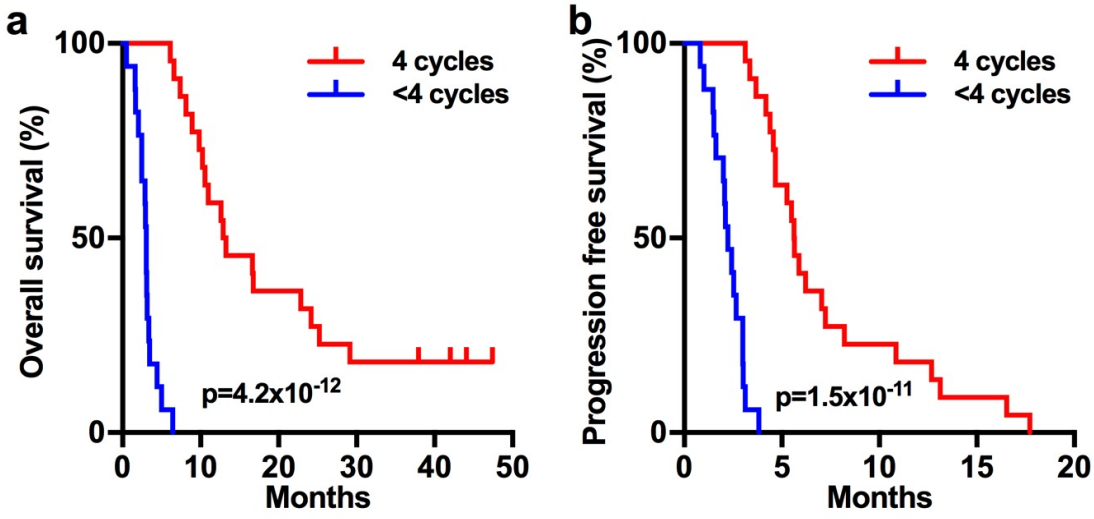
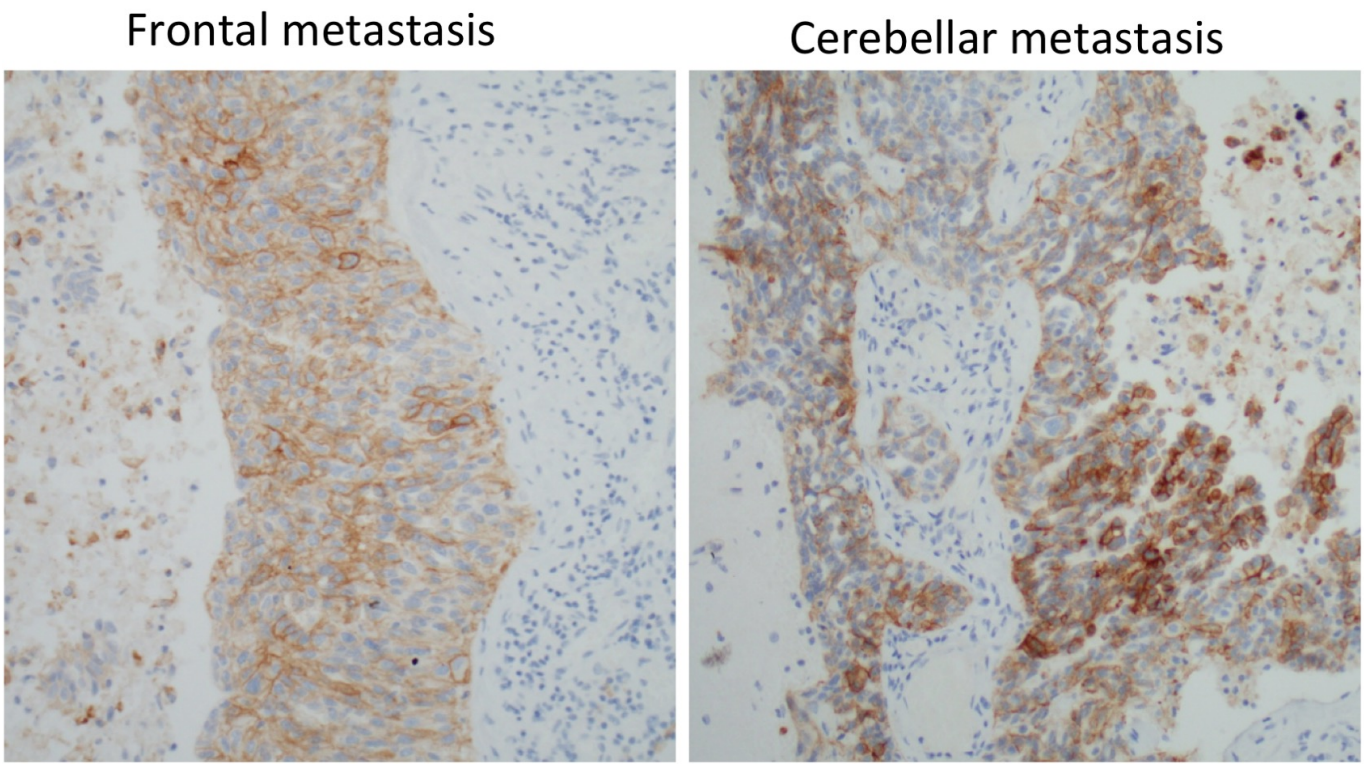
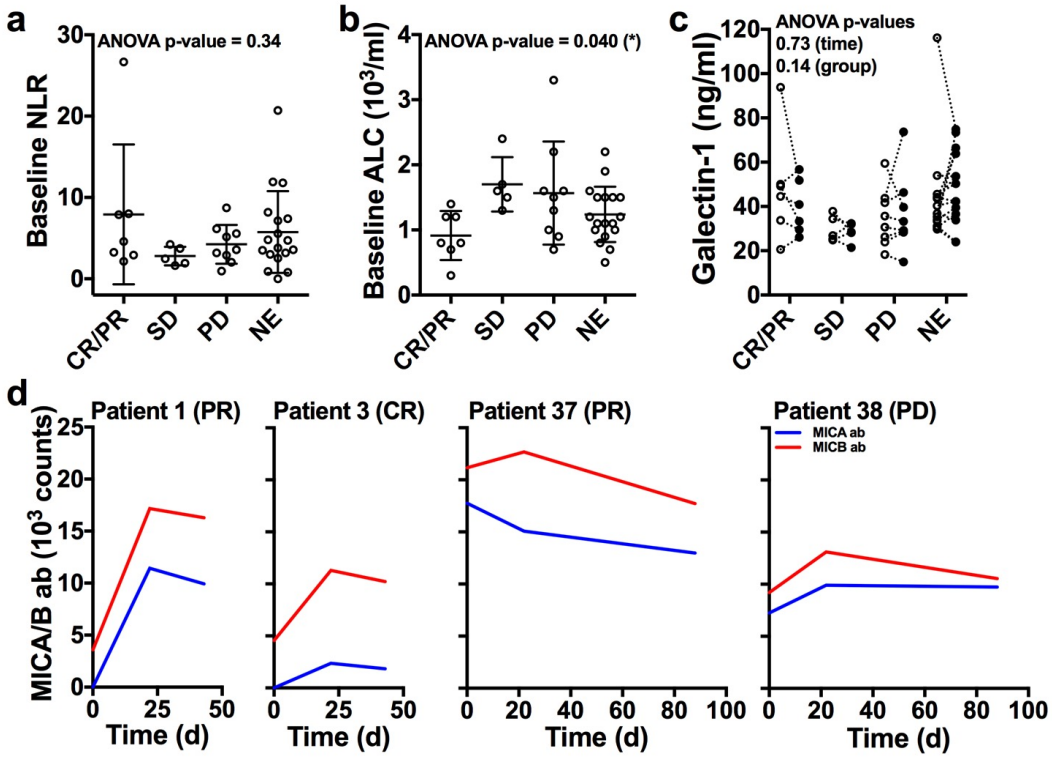
SUPPLEMENTARY INFORMATION



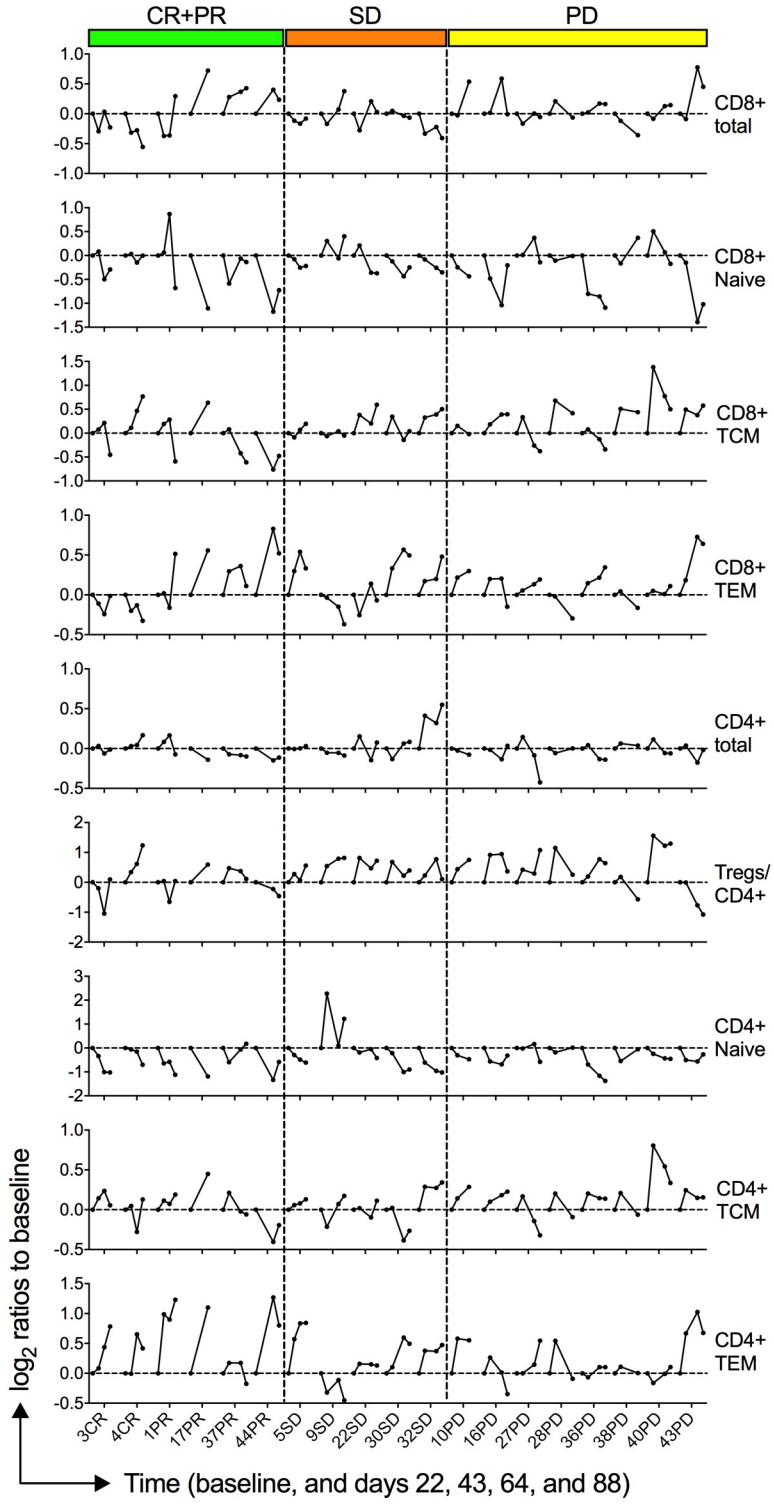
Supplementary Figure 1. Survival comparison between patients completing and not completing treatment. Comparison of (a) overall survival and (b) progression free survival between patients completing treatment ("4 cycles"; n=22) and patients that either progressed or died before completion of treatment ("<4 cycles", n=17). Statistical significance was determined using a two-sided log-rank test.



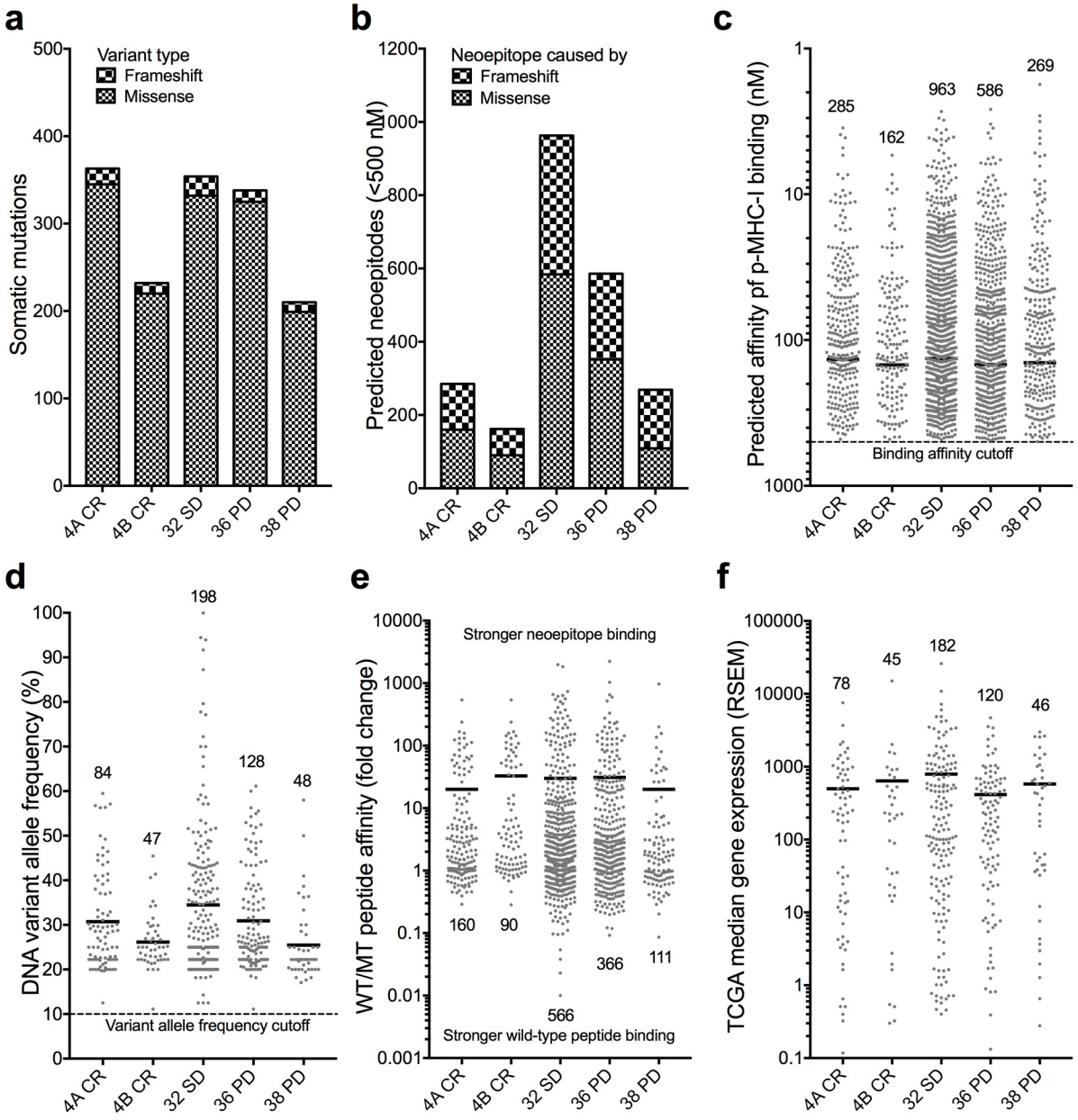
Supplementary Figure 2. Expression of PD-L1 in the two brain metastases of complete responder patient #4. The two brain metastases were surgically resected about three months prior to the patient enrollment in the trial. Immunohistochemical staining for PDL-1 was performed only once using a pre-established protocol and appropriate positive and negative controls, as detailed in Methods. Magnification 200X.



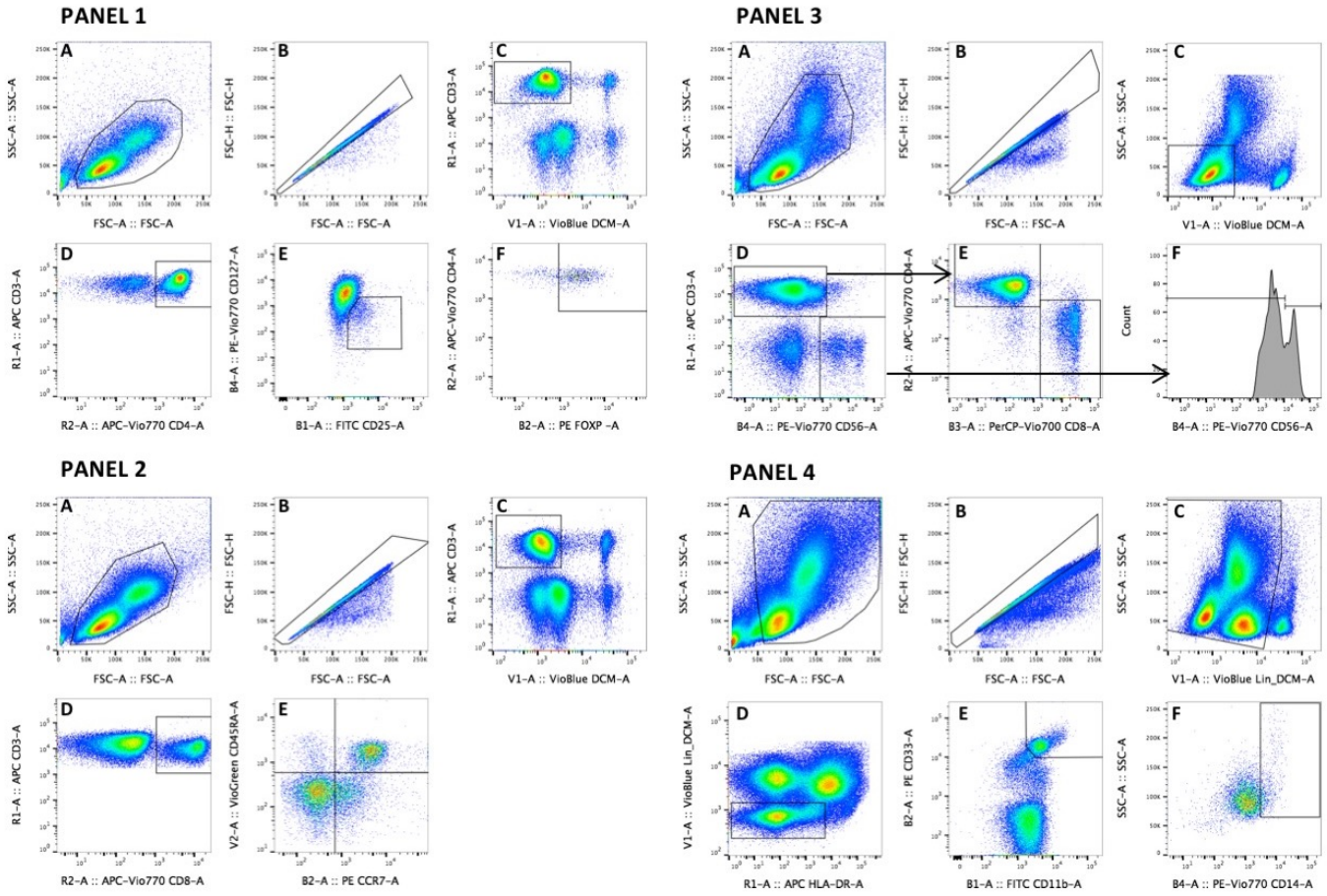
Supplementary Figure 3. Analysis of neutrophil-to-lymphocyte ratio (NLR) and absolute lymphocyte counts (ALC) at baseline, and changes in soluble markers during treatment in patients with different response to RT and ipilimumab. (a-b) Each symbol represents one patient; mean and standard deviation within response group is shown. N=7, 5, 9, and 18 patients for CR/PR, SD, PD, and NE groups, respectively, for NLR and ALC measurements. Two-sided one-way ANOVA was used to determine statistical significance of the differences between response groups. (c) Each symbol represents galectin-1 level in an individual patient measured at baseline (empty circle) and day 22 (filled circle). Data is paired (represented by dashed line) for each patient. N=6, 5, 8, and 14 patients for CR/PR, SD, PD, and NE response groups, respectively. Two-sided two-way ANOVA was used to determine statistically significant differences. (d) Examples of changes in antibodies (ab) to soluble MICA and MICB (blue and red lines represent MICA ab and MICB ab, respectively). Patients #1 and #3 showed a large and a more modest increase, respectively, in the serum levels of antibodies against sMICA and sMICB. Patient #37 had high levels of antibodies at baseline that showed a modest decrease overtime, while in patient #38 the levels were relatively stable over the course of treatment.



Supplementary Figure 4. Changes in T cell subsets during treatment. Data are shown as log2 fold change to baseline in each patient for naïve, central memory (CM), effector memory (EM) and regulatory T cells (Treg) within the CD8 and CD4 compartment, as indicated.



Supplementary Figure 5. Mutation and neoantigen landscape in NSCLC tumors prior to treatment with RT and Ipilimumab. (a) Number of missense and frameshift mutations in pretreatment tumor tissue of patients #4, 32, 36 and 38 determined by whole exome sequencing (WES). (b) Number and variant source of predicted neoepitopes from the missense and frameshift mutations (identified from the WES data using Varscan). NetMHC and NetMHCpan algorithms were used to predict neoepitopes using the pVAC-seq pipeline. A neoepitope was defined as a variant peptide binding MHC-I with an affinity < 500nM. (c) Binding affinity between the predicted neoepitopes and the MHC-I complex. Lower binding affinity equals stronger predicted binding of the neoepitope to MHC-I. (d) Variant allele frequency (VAF) of variants giving rise to the predicted neoepitopes. VAF is calculated by dividing the number of variant reads by the total number of reads for a genomic location. (e) The binding of the corresponding wild-type (WT) peptide to MHC-I was determined for each neoepitope. Then, the ratio of WT and neoepitope (MT) was calculated. Stronger neoepitope binding is predicted for neoepitopes with WT/MT ratio > 1. (f) A Lung Adenocarcinoma gene expression dataset[58] was used to determine median RNA gene expression of the corresponding gene for each predicted neoepitope. (c-f) Horizontal line represents median value and numbers above or below data-points indicate sample size for each tumor sample.



Supplementary Figure 6. Flow cytometry gating strategy and panels. Panel 1. (A) SSC/FSC dot plot to identify cells with leukocyte size and granularity. (B) FSC-H/FSC-A to exclude doublet cells. (C) CD3/DCM to identify viable T cells (D) CD3/CD4 to identify CD4+ T cells. (E) CD127/CD25 to identify CD127low/negCD25+ cells. (F) FoxP3 to identify FoxP3+ Tregs. Expression of ICOS was evaluated on Tregs and FoxP3- CD4+ T cells. Panel 2. (A) SSC/FSC dot plot to identify cells with leukocyte size and granularity. (B) FSC-H/FSC-A to exclude doublet cells. (C) CD3/DCM to identify viable T cells (D) CD3/CD8 to identify CD8+ T cells (E) CD45RA/CCR7 to identify naive T cells (T_N, CD45RA+/CCR7+), central memory T cells (T_{CM}, CD45RA-CCR7+) and effector memory T cells (T_{EM}, CD45RA-CCR7-). Expression of PD-1, TIM-3 and Ki-67 was evaluated on CD8 T cell subsets. Panel 3. (A) SSC/FSC dot plot to identify cells with leukocyte size and granularity. (B) FSC-H/FSC-A to exclude doublet cells. (C) SSC/DCM to identify viable lymphocytes (D) CD3/CD56 to identify T cells and CD3-CD56+ NK cells (E) CD4/CD8 to identify CD4+ and CD8+ T cells (F) Intensity of CD56 staining was used to identify CD56dim cytotoxic NK cells and CD56bright immunoregulatory NK cells. Expression of CD16, NKG2D and 41BB was evaluted on T cell and NK cell subsets. Panel 4. (A) SSC/FSC dot plot to identify cells with leukocyte size and granularity. (B) FSC-H/FSC-A to exclude doublet cells. (C) SSC/DCM was used to identify viable cells (D) HLA-DR was used to identify HLA-DRlow/neg cells (E) CD33/CD11b was used to identify CD33+CD11b+ MDSCs (F) CD14 was used to identify CD14+ monocytic MDSCs. FSC = forward scatter, SSC = side scatter, DCM = dead cell marker, MDSCs = myeloid-derived suppressor cells.

Supplementary Table 1. Baseline characteristics for the 39 patients entering the trial. Baseline characteristics for all patients enrolled (n=39) or grouped based on completion of treatment (n=22 patients completed treatment; n=17 patients did not complete treatment). Statistically significant differences between the two latter groups for each baseline characteristic were calculated; p-values for continuous and categorical data were calculated using Wilcoxon rank sum test and Fisher's exact test, respectively (all tests were two-sided).

Baseline characteristics (n=39)	All (n=39)	Did not complete Tx (n=17)	Completed Tx (n=22)	p-value
Age – median (range)	68 (48-97)	68 (49-97)	69 (48-87)	0.70
Gender				
Male – no. (%)	16/39 (41.0)	5/17 (29.4)	11/22 (50)	0.33
Female – no. (%)	23/39 (59.0)	12/17 (70.6)	11/22 (50)	
Histology				
Adenocarcinoma – no. (%)	34/39 (87.2)	15/17 (88.2)	19/22 (86.4)	
Squamous cell carcinoma – no. (%)	3/39 (7.7)	0/17 (0)	1/22 (4.5)	0.87
Sarcomatoid – no. (%)	1/39 (2.6)	1/17 (5.9)	0	
Neuroendocrine – no. (%)	1/39 (2.6)	1/17 (5.9)	2/22 (9.1)	
ECOG performance status – median (range)	1 (0-2)	1 (0-2)	1 (0-2)	0.10
0-1 – no. (%)	32/39 (82.0)	12/17 (70.6)	20/22 (90.9)	0.21
2 – no. (%)	7 (17.9)	5/17 (29.4)	2/22 (9.1)	
Prior therapies				
Chemotherapy – median (range)	2 (1-8)	3 (1-8)	2 (1-8)	**0.050**
≤2 – no. (%)	21/38 (55.3)	7/17 (41.2)	14/21 (66.7)	0.19
>2 – no. (%)	17/38 (44.7)	10/17 (58.8)	7/21 (33.3)	
Radiotherapy – median (range)	1 (0-4)	1 (0-4)	1 (0-3)	0.77
≤1 – no. (%)	28/39 (71.8)	13/17 (76.5)	15/22 (68.2)	0.72
>1 – no. (%)	11/39 (28.2)	4/17 (23.5)	7/22 (31.8)	
Smoking status (pack-years) – median (range)	30 (0-240)	25.8 (0-125)	35 (0-240)	0.85
≤20 – no. (%)	14/38 (36.8)	6/16 (37.5)	8/21 (38.1)	1
>20 – no. (%)	23/38 (	10/16 (62.5)	13/21 (61.9)	
NLR – median (range)	3.7 (0-26.7)	4.7 (0-20.7)	3.4 (0.8-26.7)	0.26
<4 – no. (%)	21/38 (55.3)	8/17 (47.1)	13/21 (61.9)	0.53
≥4 – no. (%)	17/38 (44.7)	9/17 (0-52.9)	8/21 (38.1)	
Metastases[a] – median (range)	3 (1-6)	4 (1-5)	2.5 (1-6)	**0.017**
Bone – no. (%)	16/39 (41.0)	11/17 (64.7)	5/22 (22.3)	**0.011**
Brain – no. (%)	16/39 (41.0)	7/17 (41.2)	9/22 (40.9)	1
Liver – no. (%)	9/39 (23.1)	6/17 (35.3)	3/22 (13.6)	0.14
Lung – no. (%)	34/38 (89.5)	15/17 (88.2)	19/22 (86.4)	1
Lymph node – no. (%)	31/38 (81.6)	14/17 (82.4)	17/22 (77.3)	1
Soft tissue or other organ – no. (%)	23/38 (60.5)	6/17 (35.3)	17/22 (77.3)	0.29
Mutational status[b]				
EGFR – no. (%)	6/29 (20.7)	2/12 (16.7)	4/17 (23.5)	1
ROS1 – no. (%)	0/6 (0)	0/3 (0)	0/3 (0)	1
RET – no. (%)	1/11 (9.1)	1/4 (25)	0/7 (0)	0.36
ALK – no. (%)	0/19 (0)	0/7 (0)	0/12 (0)	1
Kras – no. (%)	7/20 (35.0)	1/9 (11.1)	6/11 (54.5)	0.07

[a]Number of organs involved per patient.

[b]Not all genes were tested for all patients. Fraction of patients positive over the total number of patients tested.

Supplementary Table 2. Occurrence and type of adverse events (AE). Adverse events as related to Ipilimumab or radiotherapy treatment. Grade of adverse event is shown: 1 – mild, 2 – moderate, 3 – Severe or medically significant but not immediately life-threatening, 4 – Life-threatening consequences, and 5 – Death related to AE.

	CTCAE v4.0 Treatment related adverse event – no. (%)[a]									
	Ipilimumab					Radiotherapy				
	Grade									
Adverse event	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Any treatment related AE	34 (87.2)	24 (61.5)	14 (35.9)	0	1 (2.6)	12 (30.8)	9 (23.1)	3 (7.7)	0	1 (2.6)[b]
Abdominal pain	2 (5.1)	0	0	0	0	1 (2.6)	0	0	0	0
Acute kidney injury	0	1 (2.6)	0	0	0	0	1 (2.6)	0	0	0
Alkaline phosphatase	4 (10.3)	0	2 (5.1)	0	0	0	0	0	0	0
ALT	4 (10.3)	1 (2.6)	1 (2.6)	0	0	0	0	0	0	0
Anemia	2 (5.1)	0	0	0	0	0	0	0	0	0
Anemia	2 (5.1)	1 (2.6)	0	0	0	1 (2.6)	0	0	0	0
Anorexia	10 (25.6)	3 (7.7)	0	0	0	2 (5.1)	0	0	0	0
Aspiration	0	0	0	0	0	0	1 (2.6)	0	0	0
AST	2 (5.1)	1 (2.6)	2 (5.1)	0	0	0	0	0	0	0
Bullous dermatitis	1 (2.6)	0	0	0	0	0	0	0	0	0
Cardiac arrest	0	0	0	0	1 (2.6)	0	0	0	0	1 (2.6)[b]
Constipation	1 (2.6)	0	0	0	0	0	0	0	0	0
Cough	1 (2.6)	2 (5.1)	0	0	0	0	0	0	0	0
Creatinine	1 (2.6)	0	0	0	0	0	0	0	0	0
Dehydration	1 (2.6)	0	1 (2.6)	0	0	1 (2.6)	0	0	0	0
Diarrhea	8 (20.5)	2 (5.1)	1 (2.6)	0	0	0	0	0	0	0
Dry skin	1 (2.6)	1 (2.6)	0	0	0	0	0	0	0	0
Dyspnea	1 (2.6)	4 (10.3)	1 (2.6)	0	0	0	1 (2.6)	2 (5.1)	0	0
Edema	4 (10.3)	0	0	0	0	0	0	0	0	0
Fatigue	12 (30.8)	3 (7.7)	1 (2.6)	0	0	9 (23.1)	4 (10.3)	1 (2.6)	0	0
Fever	3 (7.7)	0	0	0	0	0	0	0	0	0
Headache	1 (2.6)	0	0	0	0	0	0	0	0	0
Hyperglycemia	1 (2.6)	0	0	0	0	1 (2.6)	0	0	0	0
Hypernatremia	1 (2.6)	0	0	0	0	0	0	0	0	0
Hyperthyroid	2 (5.1)	0	0	0	0	0	0	0	0	0
Hypoalbuminemia	1 (2.6)	0	0	0	0	0	0	0	0	0
Hypocalcemia	1 (2.6)	0	0	0	0	0	0	0	0	0
Hyponatremia	4 (10.3)	0	0	0	0	0	0	0	0	0
Hypophysitis	0	1 (2.6)	0	0	0	0	0	0	0	0
Hypothyroid	1 (2.6)	2 (5.1)	0	0	0	0	0	0	0	0
Hypoxia	0	2 (5.1)	0	0	0	0	0	0	0	0
Leukopenia	2 (5.1)	0	0	0	0	0	0	0	0	0
Nausea	1 (2.6)	2 (5.1)	1 (2.6)	0	0	0	1 (2.6)	0	0	0
Neck edema	1 (2.6)	0	0	0	0	0	0	0	0	0
Pain	0	1 (2.6)	0	0	0	0	0	0	0	0
Peripheral neuropathy	3 (7.7)	0	0	0	0	0	0	0	0	0
Pruritis	10 (25.6)	7 (17.9)	0	0	0	0	0	0	0	0
Rash	8 (20.5)	2 (5.1)	2 (5.1)	0	0	0	1 (2.6)	0	0	0
Seizure	1 (2.6)	0	0	0	0	0	0	0	0	0
Skin ulceration	1 (2.6)	0	0	0	0	1 (2.6)	0	0	0	0
Tachycardia	0	1 (2.6)	1 (2.6)	0	0	0	1 (2.6)	0	0	0
Thrombocytopenia	1 (2.6)	0	1 (2.6)	0	0	0	0	0	0	0
Vomiting	4 (10.3)	1 (2.6)	2 (5.1)	0	0	0	0	0	0	0
Weight loss	2 (5.1)	2 (5.1)	0	0	0	0	0	0	0	0

[a]Numbers indicate no. of patients (% of total)

[b]Possibly related grade 5 AE

Supplementary Table 3. Treatment responses for the 21 evaluable patients completing RT and 4 cycles of ipilimumab. Comparison of treatment response evaluated by RECIST, irRC, and best responding lesion for each patient.

Treatment responses (n = 21)					
Patient ID	**RT site[a]**	**RECIST[b]**	**irRC[c]**	**Best abscopal response[d]**	**RT regimen[e]**
3	Right lung	CR	CR	CR	6 Gy x 5
4	Right lung	CR	CR	CR	6 Gy x 5
1	Right paraspinal	PR	SD	PR	6 Gy x 5
17	Mediastinum	PR	SD	CR	9.5 Gy x 3
23	Left lung	PR	SD	PR	9.5 Gy x 3
37	Right lung	PR	PR	CR	9.5 Gy x 3
44	Right lung	PR	PR	CR	9.5 Gy x 3
5	Right lung	SD	SD	SD	6 Gy x 5
9	Abdominal node	SD	SD	SD	6 Gy x 5
22	Right lung	SD	SD	SD	9.5 Gy x 3
30	Liver	SD	SD	PR	9.5 Gy x 3
32	Right lung	SD	SD	SD	9.5 Gy x 3
10	Right lung	PD	PD	SD	6 Gy x 5
16	R ileopsoas	PD	PD	PD	9.5 Gy x 3
27	Liver	PD	PD	PR	9.5 Gy x 3
28	Right lung	PD	NE[f]	NE[f]	9.5 Gy x 3
33	Liver	PD	PD	PD	9.5 Gy x 3
36	Right lung	PD	PD	SD	9.5 Gy x 3
38	Left lung	PD	PD	PD	9.5 Gy x 3
40	Right lung	PD	PD	PD	9.5 Gy x 3
43	Left pelvis	PD	SD	PR	9.5 Gy x 3

[a]Site of radiation therapy (RT)
[b]Treatment response based on RECIST1.1 criteria
[c]Treatment response based on immune-related response criteria (irRC)
[d]Treatment response based on RECIST1.1 criteria but only using the best responding lesion in each patient
[e]Dose per fractionation and number of fractionations
[f]NE = Not Evaluable

Supplementary Table 4. Comparison of treatment response evaluation using different criteria for all patients (n=39) entering the clinical trial. Treatment responses (Objective Response Rate and Disease Control Rate) was calculated for the 21 patients completing treatment and for the 39 patients enrolled based on intent to treat (ITT). For RECIST response groups the breakdown between the two radiation therapy (RT) regimens used is shown.

	Treatment response rates (%)				RT regimen[e]	
	RECIST[a]	**irRC[b]**	**BAR[c]**	**irBOR[d]**	**6 Gy x 5**	**9.5 Gy x 3**
ORR[f]	7/21 (33.3)	4/20 (20)	10/20 (50)	5/20 (25)	3/6 (50)	4/15 (26.7)
ITT ORR[g]	7/39 (17.9)	4/39 (10.3)	10/39 (25.6)	5/39 (12.8)	3/13 (23.1)	4/26 (15.4)
DCR[h]	12/21 (57.1)	13/20 (65)	16/20 (80)	14/20 (70)	5/6 (83.3)	7/15 (46.7)
ITT DCR[i]	12/39 (30.8)	13/39 (33.3)	16/39 (41)	14/39 (35.9)	5/13 (38.5)	7/26 (26.9)

[a]Treatment response based on RECIST1.1 criteria
[b]Treatment response based on immune-related response criteria (irRC)
[c]BAR, Best Abscopal Response based on RECIST1.1 criteria
[d]irBOR, Best Objective Response based on irRC criteria
[e]Breakdown between the two RT regimens (dose per fraction x number of fractions) in responses as evaluated by RECIST.
[f]ORR, Objective Response Rate, number of patients with CR or PR divided with total number of patients completing the trial (21 patients)
[g]ITT ORR: ORR calculated using intent to treat (ITT), i.e., all 39 patients entering the trial
[h]DCR, Disease Control Rate, number of patients with CR, PR, or SD divided with total number of patients completing the trial (21 patients)
[i]ITT DCR: DCR calculated using intent to treat (ITT), i.e., all 39 patients entering the trial

Supplementary Table 5. Evaluation of PD-L1 expression and CD8 T cell infiltration in pretreatment tumor samples. For patients with available archival tissue, tumor sections were stained for PDL-1 and CD8 by immunohistochemistry. In most cases tissue was from a metastasis surgically resected or sampled for diagnostic purposes within <1 year before the patient enrolled in the trial.

Patient ID (n=15)	Response (RECIST)[a]	Site[b]	Year[c]	CD8 T cells per 200X field (average ± sd)[d]	PDL-1 tumor cell[e]	
					TC (%)	Interpretation
1	PR	subcutis	2014	NA[f]	>1%	POS
2	NE	lung	2011	3.7 ± 2.1	>1%	POS
3	CR	pleura	2014	NA	>1%	POS
4	CR	brain	2014	29.8 ± 17.6	>50%	POS.Hi
15	NE	mediastinal LN	2014	2 ± 0	<1%	NEG
16	PD	psoas	2014	9.2 ± 16.2	<1%	NEG
18	NE	pleura	2014	3.7 ± 3.1	<1%	NEG
32	SD	brain	2013	7.4 ± 7.1	<1%	NEG
34	NE	pleura	2015	23 ± 5.3	>1%	POS
36	PD	brain	2014	111 ± 22.6	>50%	POS.Hi
38	PD	supraclavicular LN	2014	59 ± 6.2	>1%	POS
39	NE	lung	2013	123 ± 25.2	>1%	POS
41	NE	axilla	2015	NA	<1%	NEG
43	PD	bladder	2015	78.6 ± 37.0	>1%	POS
44	PR	lung	2015	NA	>1%	POS

[a]Treatment response based on RECIST1.1 criteria. PD, progressive disease at evaluation. NE, non-evaluable patient who did not complete treatment. CR, complete response. PR, partial response. SD, stable disease
[b]Site of pretreatment tumor sample. Archival tissue was used from the most recent resection/biopsy available
[c]Year when tumor biopsy/surgical resection was performed
[d]Number of CD8+ T cells per 200X microscopy field. Values shown are average ± standard deviation (sd)
[e]PD-L1 status was determined by examination of membrane staining on carcinoma cells. Tumors were interpreted as PD-L1 positive (POS) if >1% of tumor cells (TC) expressed PD-L1 with intensity of at least 2+. Cases with >50% positive TC were interpreted as positive high (POS.Hi).
[f]NA, no available tissue

Supplementary Table 6. Comparions of baseline characteristics between patients with disease control and patients with progressive disease after completing treatment. To assess statistically significant differences between the two treatment response groups for each baseline characteristic p-values for continuous and categorical data were calculated using Wilcoxon rank sum test and Fisher's exact test, respectively (all tests are two-sided). Only the proportion of patients with EGFR mutated cancers was significantly higher in patients with progressive disease.

Baseline characteristics	Progressive disease (n=9)	Disease control (n=12)	p-value
Age – median (range)	68 (48-75)	69 (51-87)	0.27
Gender			
Male – no. (%)	3/9 (33.3)	8/12 (66.7)	0.20
Female – no. (%)	6/9 (66.7)	4/12 (33.3)	
Histology			
Adenocarcinoma – no. (%)	9/9 (100.0)	10/12 (83.3)	0.49
Squamos – no. (%)	0	2/12 (16.7)	
ECOG performance status – median (range)	1 (0-2)	1 (0-2)	0.67
0-1 – no. (%)	9/9 (100.0)	10/12 (83.3)	0.49
2 – no. (%)	0	2/12 (16.7)	
Prior therapies			
Chemotherapy – median (range)	2 (1-4)	2 (1-8)	0.94
≤2 – no. (%)	6/9 (66.7)	6/10 (60)	1
>2 – no. (%)	3/9 (33.3)	4/10 (40)	
Radiotherapy – median (range)	2 (0-3)	1 (0-2)	0.24
≤1 – no. (%)	4/9 (44.4)	10/12 (83.3)	0.088
>1 – no. (%)	5/9 (55.6)	2/12 (16.7)	
Smoking status (pack-years) – median (range)	19.8 (0-40)	38 (0-90)	0.063
≤20 – no. (%)	4/8 (50.0)	3/12 (25.0)	0.36
>20 – no. (%)	4/8 (50.0)	9/12 (75.0)	
NLR – median (range)	3.56 (0.95-8.7)	3.49 (1.6-26.7)	0.97
<4 – no. (%)	6/9 (66.7)	7/12 (58.3)	1
≥4 – no. (%)	3/9 (33.3)	5/12 (41.7)	
Metastases[a] – median (range)	2 (2-6)	2.5 (1-3)	0.45
Bone – no. (%)	2/9 (22.2)	2/12 (16.7)	1
Brain – no. (%)	5/9 (55.6)	3/12 (0.25)	0.20
Liver – no. (%)	2/9 (22.2)	1/12 (8.3)	0.55
Lung – no. (%)	9/9 (100)	9/12 (75)	0.23
Lymph node – no. (%)	6/9 (66.7)	10/12 (83.3)	0.61
Soft tissue or other organ – no. (%)	2/9 (22.2)	2/12 (16.7)	1
Mutational status[b]			
EGFR – no. (%)	4/8 (50)	0/9 (0)	**0.029**
ROS1 – no. (%)	0/1 (0)	0/3 (0)	1
RET – no. (%)	0/3 (0)	0/4 (0)	1
ALK – no. (%)	0/8 (0)	0/4 (0)	1
Kras – no. (%)	1/4 (25)	5/7 (71.4)	0.24

[a]Number of organs involved per patient.
[b]Not all genes were tested for all patients. Fraction of patients positive over the total number of patients tested.

Supplementary Table 7. Expression of PD-1 and Ki67 in T cells from patients peripheral blood collected at baseline and during treatment. Expression of PD1 and Ki67 by circulating CD4 and CD8 T cells was determined using flow cytometry. The overlap between PD-1$^+$ and Ki67$^+$ T cells within CD4 and CD8 subsets was analyzed to determine if expression of PD-1 was associated with activation.

	Average (range) [%]					
RECIST	**PD1+ (% of CD8+)**	**Ki67+ (% of CD8+)**	**Ki67+ (% of PD1+ CD8+)**	**PD1+ (% of CD4+)[a]**	**Ki67+ (% of CD4+)[a]**	**Ki67+ (% of PD1+ CD4+)[a]**
Baseline						
CR/PR (n=6)	30.5 (6.58-49.1)	7.73 (3.74-16.8)	21 (6.52-38.8)	15.62 (5.32-27.8)	6.7 (4.99-9.82)	27.2 (16.3-53.2)
PD (n=8)	27.73 (14.2-48)	4.56 (2.75-7.31)	14.18 (6.44-29.5)	18.73 (6.22-43.3)	4.24 (2.44-6.59)	18.39 (5.99-36.4)
SD (n=5)	31.48 (22.4-53.4)	3.68 (1.51-6.74)	11.19 (4.71-23.9)	17.92 (11.5-26.7)	3.35 (2.37-4.29)	12.22 (7.42-17.9)
During treatment						
CR/PR (n=7)	36.84 (5.43-53.3)	11.14 (5.28-22.1)	22.7 (6.96-46.1)	23.13 (4.91-42.7)	9.1 (4.98-19.3)	24.87 (11.3-60.4)
PD (n=8)	31.84 (9.73-64.8)	9.71 (3.31-34.8)	19.92 (8.19-48.2)	23.65 (10.5-44.6)	8.09 (4.05-32.1)	21.25 (7.48-58.9)
SD (n=5)	33.64 (19.9-57.8)	6.46 (2.4-13.3)	15.71 (5.66-39.8)	22.19 (11.6-31.9)	5.91 (3.82-8.89)	15.68 (8.89-26.5)

[a]CD4+ determined as CD8- fraction

Supplementary Table 8. HLA-typing of PBMC and tumor samples using Optitype. Whole-exome sequencing (WES) was performed on DNA from tumor and PBMC from 4 patients, and HLA-A, B, and C alleles were identified using Optitype. Patient #4 shows loss of heterozygosity at HLA-A and –C loci in both tumors.

Patient ID	Sample	HLA-A alleles		HLA-B alleles		HLA-C alleles	
4	PBMC	A*24:02	A*32:01	B*38:01		C*12:03	C*05:01
	Tumor A	A*24:02		B*38:01		C*12:03	
	Tumor B	A*24:02		B*38:01		C*12:03	
32	PBMC	A*68:01	A*02:01	B*35:03	B*44:02	C*07:04	C*04:01
	Tumor	A*68:01	A*02:01	B*35:03	B*44:02	C*07:04	C*04:01
36	PBMC	A*02:01	A*26:01	B*08:01	B*40:01	C*03:04	C*07:01
	Tumor	A*02:01	A*26:01	B*08:01	B*40:01	C*03:04	C*07:01
38	PBMC	A*24:03	A*01:01	B*51:01	B*58:01	C*07:01	C*15:04
	Tumor	A*24:03	A*01:01	B*51:01	B*58:01	C*07:01	C*15:04

Supplementary Table 9. Characteristics of the immunogenic mutation in *KPNA2* gene recognized by CD8 T cells in the post-treatment blood of patient #4. Using the neoantigen prediction pipeline shown in Fig. 4a a mutation was identified in the gene encoding for karyopherin a2 (KPNA2) that generated two neoepitopes predicted to bind to HLA-A*24:02 (p15) and to HLA-C*12:03 (p16). The mutation was expressed in both brain metastases of the patient as confirmed by RNA-seq. Although the predicted binding affinity of the mutated and wild type peptides to the respective HLA alleles was similar, CD8 T cells from post-treatment blood only recognized the mutated peptide, as demonstrated in ELISPOT assays (Fig. 4d). The CDR3 amino acid sequence of the TCR from the p15- and p16-specific CD8 T cells is shown.

Variant information		
Location	chr17:66039227	
Gene	KPNA2	
ENSEMBL ID	ENSG00000182481	
Nucleotide change	C>T	
AA change	R>C	
Sequencing data for tumor biopsy A		
WES coverage (reads)	90	
WES VAF (%)	44	
RNA coverage (reads)	218	
RNA VAF (%)	44	
Gene expression (FPKM)	27	
Sequencing data for tumor biopsy B		
WES coverage (reads)	67	
WES VAF (%)	34	
RNA coverage (reads)	56	
RNA VAF (%)	21	
Gene expression (FPKM)	10	
Neoepitope prediction		
Peptide ID	Peptide 15	Peptide 16
Mutated AA sequence	GYLCNLTWTL	YLCNLTWTL
Wildtype AA sequence	GYLRNLTWTL	YLRNLTWTL
Allele	HLA-A*24:02	HLA-C*12:03
MT affinity (nM)	130	479
WT affinity (nM)	81	384
Neoepitope reactive T cell clone		
TCRb CDR3 amino acid sequence	CASSSYGYTF	CASSWGSGANVLTF